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13. ABSTRACT ***(Maximum 200 Words)***

Methylthioadenosine phosphorylase (MTAP) is a key enzyme in the pathway which converts methylthioadenosine (MTA) into methionine and adenine. The *MTAP* gene is frequently deleted in a variety of different cancers. Our lab has found a link between loss of MTAP expression and the phenomena of methionine dependent growth, defined as the inability to grow on media containing methionine's metabolic precursor homocysteine. Thus, cells lacking MTAP seem to require excess methionine for growth. Other labs have shown that cells lacking MTAP have increased sensitivity to purine biosynthetic inhibitors such as methotrexate and 5,10-dideazatetrahydrofolate. These observations suggest that an effective two-pronged strategy could be used to eliminate MTAP negative breast cancer cells *in vivo*. Over the past year we have created isogenic breast cancer derived cell lines, one that is deleted for MTAP and one that has had it reintroduced. We have discovered that the cell line with MTAP reintroduced can now use MTA to make methionine, but is still unable to grow on media lacking homocysteine. This result suggests that MTAP deletion is not the primary cause of methionine dependent growth. These cell lines will be vital tools in our experiments over the next twelve months.

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PI - Signature 6/13/00 Date

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INTRODUCTION

Ideally, one would like to take advantage of genetic alterations in malignant tumor cells that could be used to target these cells for destruction. One such alteration found in metastatic breast cancer cells, as well as a variety of other tumor types, is loss of expression of the methylthioadenosine phosphorylase gene (MTAP). MTAP is involved in the methionine salvage pathway that converts the methylthioadenosine into methionine and adenosine (see appendix Figure 1 for metabolic pathway diagram). The *MTAP* gene is located adjacent to the *p16* tumor suppressor gene and is frequently found homozygously deleted in a variety of different cancers (1-4). Our lab has found a link between loss of MTAP expression and the phenomena of methionine dependent growth. Methionine dependence refers to the inability of certain tumor derived cell lines to grow on media containing homocysteine, a metabolic precursor to methionine (5). Thus cells lacking MTAP seem to require excess methionine for growth. Other labs have shown that cells lacking MTAP have increased sensitivity to purine biosynthetic inhibitors such as methotrexate and 5,10-dideazatetrahydrofolate (6). These observations suggest that an effective two-pronged strategy could be used to eliminate MTAP negative breast cancer cells *in vivo*. In this study we will determine the relationship between MTAP deletion and methionine dependent growth, attempt to identify conditions which maximally differentiate the growth effects of MTAP- and MTAP+ cells, and finally we will determine the frequency of MTAP deletion in primary breast tumors.

BODY

Technical Objective 1: Determine if MTAP is responsible for methionine dependent growth observed in MCF-7 breast carcinoma cell lines.

Task 1: Months 1-3. Construct MTAP expression vector and make stable isogenic MCF-7 cell lines with and without MTAP expression.

Progress: A construct was made by cloning the MTAP cDNA into pcDNA3.1, such that MTAP is now under the control of the CMV enhancer-promoter. This vector also contains the neomycin resistance gene for selection of stable transfectants. The construct, and a control construct lacking MTAP were used to make stable MCF-7 transfectomas. Twelve neomycin resistant clones were analyzed by Western blot analysis and one was selected for further study. Thus, this task has been completed.

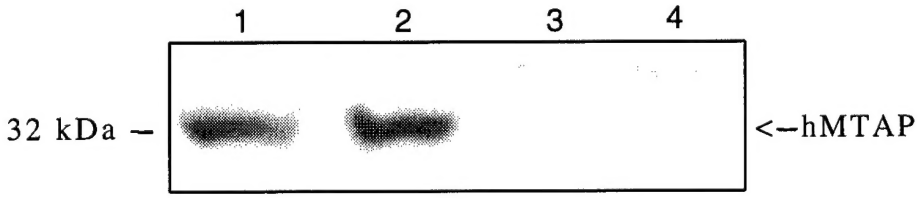


Figure 1. Western Analysis of MTAP. Western blot probed with anti-MTAP antiserum. Lane 1-Raji cell extract. Lane 2-MCF-7 cells transfected with pCR3.1 with MTAP in the sense orientation. Lane3-MCF-7 cells transfected with pCR3.1 with MTAP in the anti-sense orientation. Lane4-MCF-7 cells transfected with pCR3.1 with no insert.

Task 2: Months 4-6. Examine growth characteristics of MTAP expressing and control cells on media containing various methionine metabolites.

Progress: MCF-7 cells expressing MTAP in the sense, anti-sense, and vector alone were examined for growth rates in media containing either 100uM methionine, homocysteine (Hcy), methylthioadenosine (MTA), or 4-methylthio-2-oxobutanoate (MTOB) (see appendix). Cells lacking MTAP were unable to grow in media containing either MTA or Hcy, but grew well in Met or MTOB media. Cells with MTAP reintroduced grew well in Mer, MTOB, and MTA containing media, but still could not grow in media containing Hcy (see Table below). These results, in combination with our survey of methionine dependent cell lines, suggest that although lack of MTAP expression is common in methionine dependent cell lines, it is not the cause of the methionine dependent behavior.

Table 1. Growth behavior of MTAP transfected MCF-7 cells[a]

A)

Media (after 7 days)	pCR3.1/sense hMTAP MCF-7	pCR3.1/antisense hMTAP MCF-7	pCR3.1/CAT MCF-7
Met	100	100	100
Hcy	3	0	5
MTA	60	0	3
MTOB	88	80	98

B)

Time/Media	Raji	pCR3.1/sense hMTAP MCF-7	pCR3.1/antisense hMTAP MCF-7	pCR3.1/CAT MCF-7
3 days Met	100	100	100	100
3 days Hcy	60	4	3	5
5 days Met	100	100	100	100
5 days Hcy	56	2	1	5
7 days Met	100	100	100	100
7 days Hcy	37	2	1	0

[a]MCF-7 cells were transfected with MTAP in the sense, anti-sense, or with the vector alone. Cells were assayed for growth as described in the text. The growth values for each line are relative to the growth observed in the same cell line in the presence of methionine. A value of 0 implies there was no additional after plating. Table A shows relative growth after seven days in media containing the indicated methionine precursor. Table B shows growth after 3, 5, and 7 days compared to Raji cells, a methionine-independent cell line.

Technical objective2: Determine if MTAP deficient cell lines are at increased sensitivity for purine biosynthetic inhibitors in combination with methionine starvation.

Task 1: Months 7-9: Determine the EC-50 of the purine biosynthetic inhibitors methotrexate (MTX), L-alanosine and 5,10-dideazatetrahydrofolate.

Task 2: Months 7-9: Determine the rates of growth of MTAP expressing and non-expressing cell lines in media containing various methionine concentrations.

Task 3: Months 10-12: Determine the optimum combination of methionine restriction and purine biosynthetic inhibition which gives the largest difference in EC-50 between MTAP expressing and non-expression cell lines.

Progress: Now that we have created isogenic MTAP expressing and non-expressing cell lines we are in a position to carry out these experiments. An undergraduate summer assistant has just been hired to carrying out these studies.

Technical objective 3: Determine the frequency of MTAP expression defects in primary breast carcinoma cell lines.

Task 1: Months 6-12: Obtain primary breast carcinoma material from FCCC tumor bank and from FCCC patients.

Progress: We have obtained primary tumor material from twenty breast cancer patients treated at FCCC. Most have stage II-III disease. We have isolated protein extracts from these samples and are currently analyzing them by Western analysis.

Task 2: Months 12-18: Isolate RNA from material and assay for MTAP expression by quantitative RT-PCR.

Progress: These experiments will commence shortly.

KEY ACCOMPLISHMENTS

- Created isogenic MTAP expressing and non-expressing MCF-7 cell lines.
- Showed that MTAP expression was *not* the cause of methionine dependent growth.
- Obtained staged primary tumor material from 20 breast cancer patients.

REPORTABLE OUTCOMES

We are currently writing up this work for submission. We have created a useful cell line which we will make available to the research community after publication.

CONCLUSIONS

Based on our work we can conclude that methionine dependent growth is associated with, but not caused by defects in MTAP. However, the fact that they are commonly associated together suggests that a combination of purine inhibition and methionine starvation may be a way to selectively kill breast tumor cells.

REFERENCES

1. Zhang, H., Chen, Z. H., and Savarese, T. M. Codeletion of the genes for p16INK4, methylthioadenosine phosphorylase, interferon-alpha1, interferon-beta1, and other 9p21 markers in human malignant cell lines, Cancer Genet. Cytogenet. *86:* 22-8, 1996.

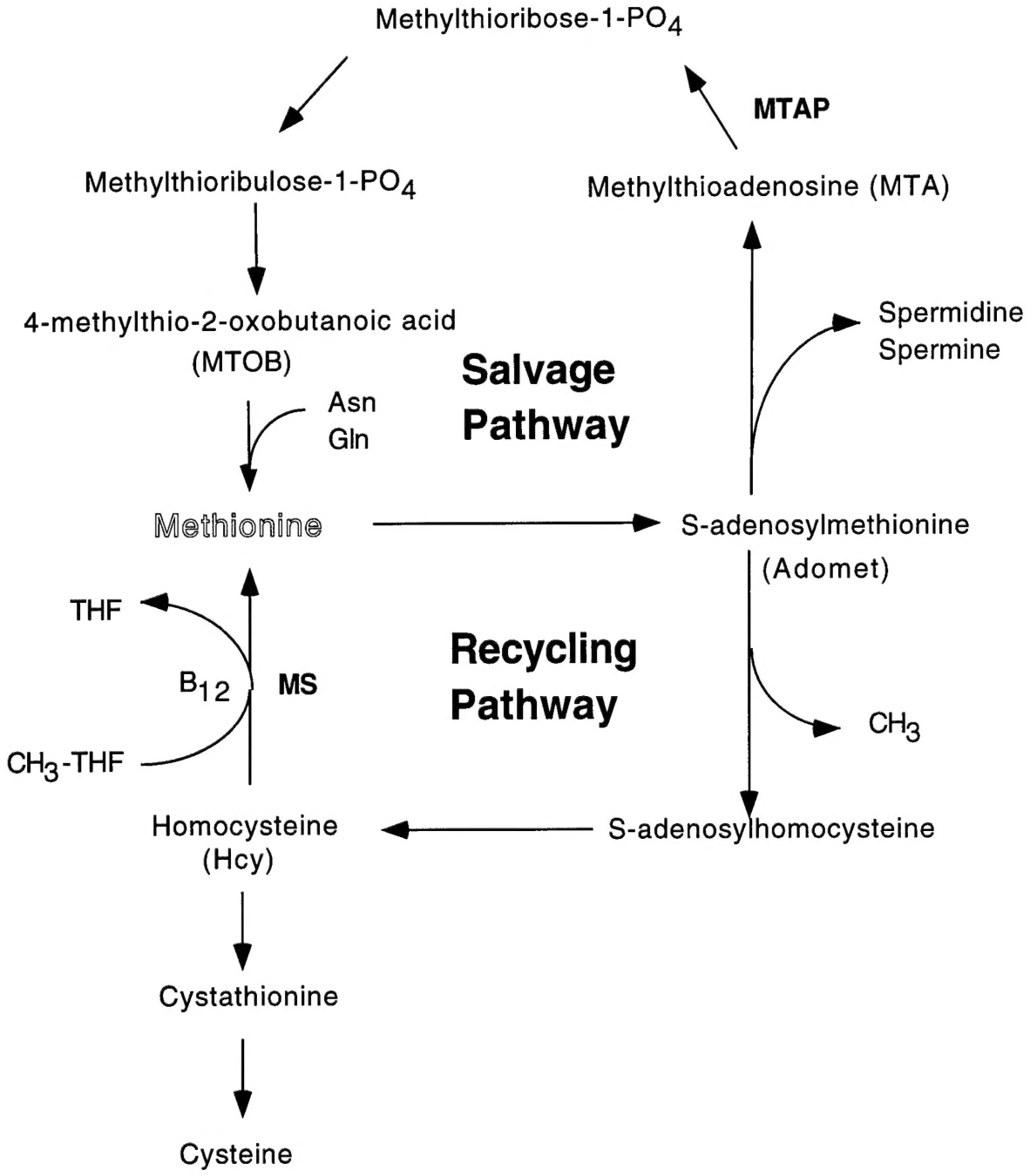
2. Stadler, W. M., Sherman, J., Bohlander, S. K., Roulston, D., Dreyling, M., Rukstalis, D., and Olopade, O. I. Homozygous deletions within chromosomal bands 9p21-22 in bladder cancer, Cancer Res. *54:* 2060-3, 1994.

3. Schmid, M., Malicki, D., Nobori, T., Rosenbach, M. D., Campbell, K., Carson, D. A., and Carrera, C. J. Homozygous deletions of methylthioadenosine phosphorylase (MTAP) are more frequent than p16INK4A (CDKN2) homozygous deletions in primary non- small cell lung cancers (NSCLC), Oncogene. *17:* 2669-75, 1998.

4. Hori, Y., Hori, H., Yamada, Y., Carrera, C. J., Tomonaga, M., Kamihira, S., Carson, D. A., and Nobori, T. The methylthioadenosine phosphorylase gene is frequently co-deleted with the p16INK4a gene in acute type adult T-cell leukemia, Int. J. Cancer. *75:* 51-6, 1998.

5. Hoffman, R. M. Altered methionine Metabolism and Transmethylation in Cancer, Anticancer Res. *5:* 1-30, 1985.

6. Hori, H., Tran, P., Carrera, C. J., Hori, Y., Rosenbach, M. D., Carson, D. A., and Nobori, T. Methylthioadenosine phosphorylase cDNA transfection alters sensitivity to depletion of purine and methionine in A549 lung cancer cells, Cancer Res. *56:* 5653-8, 1996.


Methylthioribose-1-PO4
MTAP
Methylthioribulose-1-PO4
Methylthioadenosine (MTA)
4-methylthio-2-oxobutanoic acid
(MTOB)
Asn
Gln
Salvage
Pathway
Spermidine
Spermine
Methionine
S-adenosylmethionine
(Adomet)
THF
B12
MS
CH3-THF
Recycling
Pathway
CH3
Homocysteine
(Hcy)
S-adenosylhomocysteine
Cystathionine
Cysteine